A multifactorial evaluation of different reproductive rhythms and housing systems for improving welfare in rabbit does

A multifactorial evaluation of different reproductive rhythms and housing systems for improving does welfare

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ABSTRACT

The most widespread reproductive rhythm in rabbit does rearing is based on artificial insemination (AI) performed around 10 d after kindling, resulting in high production rates, but with a high yearly replacement of animals, that are unable to sustain the enormous energy demand. The consequence is an energy deficit causing a reduction of doe reproductive career. Moreover, housing systems currently applied do not allow animals to express the typical behavioural pattern of the species, with presence of abnormal behaviours that contribute to reduce welfare. Thus, the aim of the present study was to

evaluate the best combination between two different housing systems (Standard vs Combi cage) and three reproductive Rhythms (RRs), to improve does welfare through a multiple indicators (performance, behaviour and corticosterone assessments). A total of 75 nulliparous rabbit does of Grimaud hybrid maternal line were randomly housed into 2 different systems, Standard cage (C) vs Combi cage (CC), within which 3 RRs (Intensive, I, with AI 11 days postpartum, *vs* Alternating, IE, with AI Alternated between 11 days postpartum and 30 days postpartum *vs* Extensive, E, with AI 30 days postpartum) were applied, for six consecutive reproductive cycles, following a multifactorial balanced experimental plan (2x3), as follows: CI (5 does) vs CIE (5 does) vs CE (5 does) vs CCI (20 does) vs CCIE (20 does) vs CCE (20 does). At main critical phases (AI, kindling and lactation peak) reproductive and behavioural evaluations were carried out, as well as hormonal level assessment (salivary corticosterone). RR strongly affected reproductive performances with the highest number of live-born kits ($P < 0.02$) and weight of weaned litter ($P < 0.01$) in CIE group, together with the lowest pre-weaning mortality ($P < 0.04$). The housing system also influenced behaviour: C does showed the highest frequencies of self-grooming ($P < 0.01$), feeding ($P < 0.001$) as well as stereotypes (sniffing and biting bars, $P < 0.01$), indicative of frustration for the lack of enough stimuli and consequent boredom. Corticosterone level resulted to be 3 times higher ($P < 0.01$) in the CC system. From these results, we can assume that AI after kits weaning was the best RR in Combi cage and the Alternating rhythm in Standard cage, permitting to restore fat deposits in time to sustain energy requirements, resulting in high productive performances along consecutive cycles. Whereas, from an ethological point of view, in C cage problems related to the small size of the housing system still remain.

Keywords:

Rabbit does, innovative housing system, reproductive rhythm, behaviour, welfare, corticosterone

1. Introduction

In wildlife conditions, rabbit (*Oryctolagus cuniculi* L.) is a gregarious animal and the social unit consists of an average of 2-9 females, 2-3 bucks and their progeny (Surridge et al., 1999). In commercial farm conditions, breeding does are individually housed, in standard wire cages (50–60L × 35-40H cm) with an external nest, which the doe prepares with straw materials provided by the farmer and hair from her body (Canali et al., 1991; Hudson et al., 2000).

The commercial breeding system is well adapted to cycled production, but it does not take into account neither the welfare (EFSA, 2005) nor the reproductive physiology of the doe, with reduction of fertility along cycles and high annual replacement of females (Cervera et al., 1993). In fact, the most common reproductive rhythm is intensive, based on Artificial Insemination (AI) performed 11 days *postpartum* and on weaning of the litter at about 28–30 days of age.

Regarding housing system, the European Food Safety Authority (EFSA) published a scientific opinion on the impact of housing systems on health and welfare of farmed rabbits (EFSA, 2005), highlighting several problems due to space restriction and housing practice. Several solutions have been proposed for breeding group growing rabbits (Dal Bosco et al., 2000, 2002; Trocino et al., 2004; Mugnai 2008, 2014); whereas only a few studies have been carried out on does and kits (breeding group pen, Stauffacher, 1992; double height cage and two-floor cage, Finzi et al., 1996, Berthelsen, 2000, Mirabito, 2003, 2004; colony cage, Dal Bosco et al., 2004; Mugnai et al., 2009; Dal Bosco et al., 2019) or on the semi-group housing where does are put together for a specified amount of time during the reproductive cycle (Andrist et al., 2012). Unfortunately, even if group-housing would permit to better satisfy animals needs, some critical aspects (aggression and a high kit mortality due to nest box competition among females) negatively affected productivity (Rommers et al., 2006). Moreover, Dal Bosco et al. (2019) reported a lower fat depot in does housed in colony cages compared

to standard cage, due to a higher energetic output thanks to a greater possibility of movement that further negatively affected reproductive performance of does.

Currently, housing systems have to be consistent with animal welfare regulations proposing higher space allowance (EFSA, 2005), but considering that cycled production of rabbits is efficient thanks to low space availability that permits to save energy for performance. It appears obvious that improving space allowance and consequently motor activity, energy balance, that is already a critical point in doe cycled conduction (Feugier et al., 2005), will become even more the limiting factor of the reproductive performance of does if not properly supported with a new reproductive protocol.

For all these reasons, our research aimed at evaluating the best combination between housing system (innovative (Combi) *vs* conventional cage) and reproductive rhythm (intensive vs alternating *vs* extensive), through a multifactorial evaluation (physiological, behavioural, reproductive parameters), to determine the best management practice for breeding does.

2. Materials and Methods

2.1. Animals and experimental design

The study was performed at Cascina Campora commercial farm in Buttigliera d'Asti, Italy, from October 2018 to November 2019. All animals were handled in accordance with the Turin University Bioethics Committee recommendations (Prot. N 256053 of 4/07/2017).

A total of 109 77-days old rabbit does from Grimaud hybrid maternal line, homogenous for genetic strain and age, were randomly divided into two different housing systems:

- 60 nulliparous does in Combi cage (WRSA, Combi 6®, CC), bought from a commercial company (Meneghin S.r.l, Povegliano TV, Italy), where the main characteristics of the cage were: the dimensions (97.5L x 113H cm), the plastic platform and the absence of a top cover; mobile walls,

which can allow the group housing of 4 does with their kits. The CC, was considered as an autonomous and independent production unit (Figure 1); in our trial we decided to test the CC with mobile walls in.

- 15 nulliparous does in Conventional cage (C), with dimensions of 60L x 35H cm and an external nest box. The single cage was considered as an autonomous and independent production unit.
- the remaining 34 were used as replacement.

Nulliparous does were weekly weighted, from their arrival until 133th day, to control weight gain to the first artificial insemination (AI at live weight of 4060.61 $\pm$ 334.15 g at 19th week).

At second AI, both CC and C does were divided into three different experimental subgroups, according to 3 reproductive rhythms:

- Intensive (I): AI 11 days *postpartum;*
- Alternating (IE): AI 11 days *postpartum*, alternated with AI after weaning (30 days *postpartum*);
- Extensive (E): AI performed after weaning (30 days *postpartum*).

The corresponding balanced (2x3) experimental plan, was composed by:

- CCI group = 20 CC does submitted to an Intensive rhythm
- CCIE group = 20 CC does submitted to an Alternating rhythm
- CCE group = 20 CC does submitted to an Extensive rhythm
- CI = 5 C does with an Intensive rhythm
- CIE = 5 C does with an Alternating rhythm (second AI 11 days *postpartum*, third AI insemination after weaning (30 days *postpartum*)
- CE = 5 C does with an extensive rhythm.

113 All not pregnant animals were re-inseminated after manual palpation (12 days after AI) following the
114 corresponding initial rhythm.

115 Rabbits were raised with daily control of the environmental temperature (15-18 °C) and relative
116 humidity (60-75%). The barn was artificially ventilated (0.3 m/sec) and animals were submitted to
117 natural photoperiod. The animals received a commercial diet containing 16.9% crude protein, 14.2%
118 crude fibre, and 3.5% ether extract.

119 The colony effect was not investigated because we decided to first compare the greater availability
120 of space of the Combi system, compared to the smaller control cage, focusing on the evaluation of
121 different reproductive rhythms combined with the two type of housing.

122

123 *2.2. Reproductive and productive performances*

124 AI was performed in the morning by inseminating 0,5 mL of diluted fresh semen, containing
125 about 10 million spermatozoa (Castellini and Lattaioli, 1999). No estrus synchronization was done.
126 Ovulation was induced by inoculating 0,2 mL/doe of intramuscular lecirelin (Dalmarelin, Fatro®,
127 Italy). Manual palpation to assess pregnancy was performed 12 days after AI. The following
128 reproductive/productive indexes were recorded: does weight at AI, sexual receptivity (SR: vulva color
129 and turgescence: a doe was deemed receptive when its vulva was red or purple and turgid), fertility
130 rate (F: kindling/inseminations x 100); at kindling we recorded number of live born (LK) and dead
131 kits/litter (DK), weight of weaned litter and individual weight at weaning (weaned kits total weight
132 /total number of weaned kits) and pre-weaning mortality (dead kits/number of weaned kits).

133 The indexes of efficiency were calculated in terms of: overall productivity (number and weight of
134 rabbits sold/year/doe), production losses (difference between actual and theoretical production

135 considering fertility rate = 100, mortality of the young rabbits = 0 and kindling interval = 60) and
136 efficiency of the system (Castellini et al., 2005).

137 Controlled nursing was performed until 16 day after birth by permitting the does access to the nest
138 only once a day for 15 min (Castellini et al., 2003).

139

140 *2.3. Body Condition Score*

141 Body Condition Score (BCS) was attributed at each AI. The scoring was based on feel by
142 hand loin and rump regions. The loin was felt for vertical bone protrusions (spinous process) and
143 fullness of muscle over and around the vertebrae, and the rump for bone protrusions and fullness of
144 muscle. The loin was subjectively evaluated according to poor, intermediate or wide level, whereas
145 the rump for poor or wide level. The body condition was scored "0" if loin was poor; "1" if loin was
146 intermediate and rump was poor; "2" if loin was intermediate or wide and rump was wide (Bonanno
147 et al., 2008).

148

149 *2.5. Behavioral recordings*

150 Behavioral patterns of 39 rabbit does (10/rhythm in CC system and 3/rhythm for C system)
151 were recorded through direct observations by two experienced operators who had been previously
152 trained together, in order to guarantee an accurate inter-observer agreement (Cohen,1960), following
153 the Focal Animal Scan Sampling Method (Martin and Bateson, 1986) during all reproductive cycles,
154 in three significant moments: 2 days after artificial inseminations, 2 days after birth and 2 days after
155 lactation peak for a total of 6 days/cycle/group. Data were reported on a designed table. Before each
156 observation, 5 minutes were allowed to the animals for adaptation to the presence of operators. To

157 establish the end of a performed behaviour, 10 sec were allowed to determine if the same behaviour
158 was repeated; after this time, a new behaviour was recorded (Bornett et al., 2000).

159 To develop the ethogram (Table 1), the following behaviours were recorded: static activities
160 (crouching, staying, sitting up, lying down), moving features (running, exploring, jumping), self-
161 grooming, alert (standing-up), presence of stereotypies (sniffing and biting bars), maternal attitude
162 (quality of the nest), feeding, drinking, use of the elevated platform (only for Combi cages).

163 The inter-observer reliability index (Cohen's kappa) was assessed at 0.97. For all the experimental
164 groups, does were observed for a total of 4212 min (117 min/day x 6 days of observations x 6
165 reproductive cycles). For each animal the percentage of a particular behavior was calculated as the
166 number of times it occurred divided by the total number of observations and multiplied by 100.

167 At kindling, the quality of the nest was evaluated, through a qualitative analysis (Blumetto et
168 al., 2010), in which were assessed: level of mixing (1: no evidence of mixing between the material
169 and the doe's hair; 2: an important level of mixing between them; 3: almost the totality of the material
170 was mixed), hair (1: there is no hair in the nest; 2: it is observed that more than 50% of the nest has
171 the material visible; 3: it is observed that more than 50% of the nest has the material invisible; 4: only
172 hair can be seen) and material, in which the preservation of the original material was assessed (1: less
173 than 30% of the original material is kept; 2: between 30% and 60% of the original material is kept;
174 3: more than 60% of the original material is kept).

175

176 *2.6. Tonic immobility test*

177 The tonic immobility test was performed on 39 rabbit does (10/rhythm in CC system and
178 3/rhythm for C system) and repeated on the same animals in three moments of each reproductive
179 cycle: insemination, kindling and peak of lactation. The operator took the rabbit out of the cage and

induced immobility by turning the animal on its back and onto his arms. The immobile rabbit was laid down on a plastic support (Ferrante et al., 1992). A maximum of three attempts were carried out to induce immobility and animals were left in the immobility condition for not more than 10 minutes. The number of attempts necessary to induce immobility and the total duration of the condition were recorded for each doe.

2.7. Corticosterone samples collection and hormonal evaluation

To assess animal welfare, physiological indicators as saliva corticosterone level evaluation was performed. Before the start of the experimentation, two collection days were carried out to have baseline hormone levels. Saliva was collected using saliva collectors ® (Sarstedt SRL – Verona, Italy).

During the trial, samples were collected for 6 consecutive cycles in the same does that were submitted to TI, at three different phases: 1 day after AI, 1 day after kindling and 1 day after lactation peak (16 d after birth). After being collected, samples were immediately frozen and stored at – 80 °C until laboratory analysis. Subsequently, stored samples were thawed and prepared for hormonal assays: were centrifuged for 2 minutes at 1000 x g and, after centrifugation, recover saliva was diluited 1:4 with Assay Buffer (Arbor Assays, Ann Arbor, US) prior running in the assay. The corticosterone present in saliva samples was determined using a multispecies commercial enzyme immunoassay kits (K014; Arbor Assays, Ann Arbor, MI) validated for saliva and other biological substrates. Inter- and intra-assay coefficients of variation were <10%. According to the manufacturer, the corticosterone kit presents the following cross reactivity: 100% with corticosterone, 18.9% with 1-dehydrocorticosterone, 12.3% with Desoxycorticosterone and 0,38 with cortisol. Serial dilutions (1:4,1:8,1:16, and 1:32) of saliva samples were assayed to test for parallelism against the standard

curve (P<0.05 for all assays). The mean recovery rate of corticosterone added to saliva samples (n=6) was 96.8%. The results are reported as nanogram (ng) of corticosterone on ml of saliva.

2.8. Statistical analysis

Statistical analysis were conducted using SPSS 16.0 software package, using two-way analysis of variance (ANOVA), considering the effects of housing system, reproductive rhythms and their interactions. Since no differences were found among the three physiological phases and cycles, all the data were pooled to obtain a mean value. Data are presented as mean ± SE. Multiple comparisons of the means were carried out with LSD using Duncan test. Statistical significance was assessed as significant when $P < 0.05$.

3. Results

3.1. Reproductive performances

No significant differences were recorded regarding animals' weight, BCS and sexual receptivity (Table 2). Alternating and Extensive C cage showed the highest fertility rate, as well as Extensive CC (P <0.04). The number of weaned kits resulted to be better ($P < 0.04$) in does reared in conventional cages with Intensive and Alternating reproductive rhythms, which reflected in a higher ($P < 0.01$) weight of the litter at weaning and consequently better ($P < 0.03$) individual weight of each kit. Moreover, regarding pre-weaning mortality, CC group showed the worst ($P < 0.04$) results.

224 The indexes of global productivity (Table 3) showed that C group under the intensive reproductive
225 rhythm produced the highest number and weight of rabbits sold/year/doe ($P < 0.05$), with the lowest
226 production losses (kg) among groups, but with the highest doe replacement rate ($P < 0.05$). On
227 contrary, CC does submitted to an extensive rhythm showed high productive performances with the
228 lower incidence of animals replaced but less kindling/year as a consequence of greater kindling
229 interval due to the applied reproductive rhythm ($P < 0.05$).

230 *3.2. Behavioural patterns*

231 In Table 4 are presented behavioural welfare results. Static activities were the most frequent behaviors
232 performed, with higher values in CC does for crouching ($P < 0.04$) with erect ears, while lying down
233 ($P < 0.03$) with ears down was more performed in C housing. CI does showed the higher percentages
234 in terms of feeding ($P < 0.001$) and drinking ($P < 0.002$) as well as the worst results in terms of biting
235 ($P < 0.01$) and sniffing bars ($P<0.01$). Standing up with ears down resulted to be performed only in
236 conventional cages ($P < 0.02$), with higher values in intensive standard cage. Moving features were
237 not affected neither by housing system nor by reproductive rhythm, except for jumping ($P < 0.04$)
238 only performed in alternative cages due to presence of the platform. Self-grooming was performed
239 significantly more in CI group ($P < 0.01$).

240 The tonic immobility test resulted not significant between CC and C groups. Behaviours on platform
241 were only performed in CC cages, due to the absence of this structure in conventional housing.

242 The quality of the nest resulted to be significant only for the level of mixing between the hair and the
243 material supplied by the farmer for both group ($P < 0.006$) and rhythm effect ($P < 0.05$), resulting in
244 greater values within the CCIE does.

245

246

247 *3.3. Salivary corticosterone*

248 Effects of housing system, reproductive rhythm and their interactions are displayed in Table 5. No
249 significant differences were recorded in corticosterone level (ng/ml) regarding the rhythm effect and
250 interactions, while the group strongly affected ($P < 0.007$) hormone levels with values approximately
251 3 times higher in does caged in CC housing.

252

253 **4. Discussion**

254 Reproductive performances of females are influenced by sexual receptivity, lactation stage and order
255 of birth (Theau-Clément, 2007). Lactation requires a great amount of disposable energy and it's
256 closely dependent from some variables, such as fecundity. In particular, nulliparous does seems to be
257 more affected by energy deficit than multiparous, as they have to support also growth till adult age
258 (Pascal et al., 2013). In our trial, reproductive performances were not affected by cage type and
259 reproductive rhythm concerning body weight, Body Condition Score and sexual receptivity. While,
260 as expected, the extensive rhythm improved fertility rate of rabbit does, resulting in higher values for
261 both housing systems, as reported in a previous work of Theau-Clément et al. (2016), where females
262 subjected to a less intensive rhythm showed the best results in terms of fertility, litter size and number
263 of live born kits, so inseminating after kits weaning can be a possibility to improve the percentage of
264 pregnancies, thanks to an improvement of the body status with a consequent longer reproductive
265 career and also to a peak of sexual receptivity physiologically showed after weaning of the litter
266 (Theau-Clément et al. (1990). Inseminating does after weaning seems to eliminate the hormonal and
267 energetic antagonism between lactation and pregnancy and animals compensate for the lower
268 production efficiency with a higher fertility rate than conventional intensive rhythm (79.0% vs.
269 60.2%) (Castellini et al., 2003). Body conditions of does has a fundamental role, being them

responsible for their reproductive efficiency especially in terms of fertility and productivity (Naturil-Alfonso et al., 2017). This statement is also supported by the higher productivity (rabbit/sold/year/doe and live weight sold/year/doe) reached in CCE rabbit does, with the minor replacement of animals among groups (20% vs 130% of intensive rhythm in C group), without however reaching values of the intensive rhythm in conventional cage. The CE group showed worst productive performances, probably due to an increase of stressors for the smaller space available in the cage. The only disadvantage of inseminating after weaning could be that the prolongation of the fertilization-conception interval could cause an excessive increase in weight, with a consequent lower conception rate with the progression of reproductive career (Cerolini et al., 2015). CC cages showed the worst results in terms of pre-weaning mortality and weight of weaned litter, in accordance with Mikó et al. (2012) positive correlation of cage enlargement and greater height with mortality of rabbits weaned at around 30 days of age. CC cages showed different reproductive performances, but all the three groups analyzed did not reach the productivity of intensive rabbit housing. Regarding the reproductive rhythm effect on reproductive performances among groups, it should be noted that the alternate type of *postpartum* in C cage, in comparison with the fixed intensive insemination at 11 days after kindling, seems to be more adapted to the doe physiology, with a fertility rate close to 90% and reflection in the highest number of live born kits and weight of weaned litter, as well as the lowest pre-weaning mortality compared to all the experimental groups. This evidence is supported by a previous work of Castellini et al. (2003) where an improvement of reproductive parameters were recorded with a semi-intensive reproductive rhythm (alternating AI at 1 day postpartum with AI performed after kits weaning), reducing energy deficit of females of 6,7% compared to standard 11 days post kindling AI and the replacement rate of about 10%. Extensive rhythm in both housing systems showed intermediate results, with higher fertility rates but lower number of weaned litters. The combination intensive/alternating plus CC housing resulted in the lowest reproductive performances.

295 Regarding the behavioural repertoire expressed, in accordance with Gunn and Morton (1993), static
296 activities were the most common activities performed in all 6 experimental groups, probably because
297 direct observations were recorded only during the light period, between 8.00 a.m. and 16.00 p.m.,
298 during which they are usually less active being crepuscular animals (Jilge and Hudson, 2001). No
299 significant differences were observed for behavioural pattern between the different reproductive
300 rhythms of the CC system, while regarding the 3 C groups, the intensive one showed higher
301 frequencies in terms of standing up, self-grooming, stereotypies, feeding and drinking. Regarding
302 stereotypies, CI cage showed the highest percentages in terms of biting and sniffing bars, instead in
303 CC system there was a lower incidence of abnormal behaviours, confirmed by Verga et al. (2007)
304 which observed more stereotypes in individually housed rabbit does. These disorders are indicators
305 of anxiety (Poderscek et al., 1991) and usually substitute the normal behaviours which are inhibited
306 by the lack of eliciting stimuli and enough space (Gunn-Dore and Morton, 1993). According to Gunn-
307 Dore (1997), the extreme boredom induces animals to eat a greater amount of feed as observed in our
308 trial for the intensive control cage. The use of enrichment material, such as the platform available in
309 the innovative system, can lead to distraction from this abnormal behaviour. Trocino et al. (2019)
310 showed that the use of an elevated platform allows rabbits to move more, to rest in a more comfortable
311 position and to increase the exploratory behavior, without modifying the production performances.
312 However, there is often a simultaneous increase in injured rabbits which may discourage their use.
313 Similarly, an improvement of rabbit welfare conditions was found using enrichment materials such
314 as wooden bars to gnaw or PVC tubes (Marìa et al., 2005). Our results showed that when a platform
315 is available, rabbit does jumped on it and spent around 20% of their time resting there. Additionally,
316 in a previous work, Hansen and Berthelsen (2000), demonstrated that rabbit does in conventional
317 cages are more bored, showing higher comfort activities and bar biting percentages compared to does
318 reared in innovative cages with presence of an elevated platform.

319 Jumping was visible only in CC cages due to a greater availability of space, while in the C system it
320 was not impossible due to the lack of proper dimensions: this situation usually leads to a frustration
321 state, confirmed by the higher presence of sniffing and bars biting in this type of housing (Gunn-
322 Dore, 1994). As reported by Morton et al. (1993), it is fundamental to use sufficiently high cages (75
323 cm) to allow the rabbit to sit upright without its ears touching the top of the cage. In our trial, high
324 frequencies of standing up with ears down due to an insufficient cage height (35 cm) were observed
325 in C cages. However, the relevance of this behaviour as indicator of wide or poor welfare can be
326 questionable in commercial farm conditions, due to the lack of predators (Princz et al. 2008).
327 According to the same author, the commonly height utilized in rearing fattening rabbits (35-40 cm)
328 is satisfactory. In our study, animals caged in C system and CC system (113 cm) spent a percentage
329 of time in standing alert significantly different, with rabbit does housed in conventional housing most
330 frequently ($P < 0.02$) observed in this position. The evaluation of the nest showed significant
331 differences ($P < 0.05$) as regards the level of mixing. This maternal behavior was observed with a
332 greater frequency in CC rabbit does, especially in those subjected to an alternating reproductive
333 rhythm: this result is in agreement with several studies that state that high levels of stress cause
334 alterations of the maternal behavior with consequent acts of cannibalism or, as in the present study,
335 poor or non-construction and care of the nest (Verga et al., 1978; Gonzáles-Redondo and Zamora-
336 Lozano, 2008). All behavioral results were found to be extremely accurate, confirmed by Cohen's
337 kappa reliability index that reached a value of 0.97, in a range where 0 indicates no accuracy and 100
338 the maximum.

339 The tonic immobility test showed no significant differences among groups. This could be due to the
340 usual manipulation of rabbits by the farmer during the management "routine", in accordance with
341 several studies where the state of fear resulted strongly decreased when the animals got used to the
342 presence and contact with human beings, thus improving their general welfare with positive effects
343 on production performances and health status (Kersten et al., 1989; Jiezierski and Koneca, 1996).

Corticosterone salivary concentration was strongly affected by the housing system, with much higher values (almost 3 times more) in CC cages. Although this results are in disagreement with most of the previous literature (Koolhaas et al., 1993; Koob and Heinrichs, 1999; Cornale et al., 2016), this could be explained by the presence of a greater quantity of stimuli in the innovative system: the greater space available in CC housing allows a higher body exposure of animals due to the lower stocking density and this can lead breeding does to react with an anti-predator reaction (physiological stress response), feeling themselves more vulnerable than in smaller conventional cages (Monclus et al., 2006), with a consequent raising of corticosterone concentrations. Under stress, the activation of the HPA axis, together with the activation of the sympathetic-adrenomedullary system, contributes to the mobilization of energy necessary to cope with the stressful situation (Boissy 1995; von Holst 1998), with negative consequences on the ability of supporting lactation and pregnancies requirements. Moreover, Szendro et al. (2012) reported results similar to ours with higher faecal corticosterone in rabbit does housed in colony with wider space available compared to does in single housing. In our trial, the greater levels of salivary corticosterone measured in females reared in CC cages may signal the possibility of more stressful conditions for rabbits housed in wider systems, with observation of lower reproductive performances. Another factor to take into consideration is that saliva samples collection was performed only during the daylight following behavioural recordings, so values may not completely reflect the behaviour of the entire 24h. Anyway, whether this stress condition comes from the housing system per se (Combi system vs conventional cages) or could be attributed to other external factors needs to be further elucidated.

Conclusions

Given the results, we can indicate that AI after kits weaning resulted to be the best reproductive protocol in Combi cages and the Alternating rhythm in standard cage. In fact, these reproductive

protocols, thanks to an improvement of the body status, better match the compromise between the productivity and the physiological welfare of the rabbit doe.

From an ethological point of view, standard housing reduces animal behavioural freedom and also constantly leads to stereotypes, but despite this animal physiological stress seems to be lower.

Further studies are required to understand properly what combination of reproductive rhythm and housing system can completely satisfy both ethological and physiological needs, in any case keeping the farm remunerative in terms of production and efficiency.

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